CHAPTER 1: INTRODUCTION

Psoriasis is a general chronic skin disorder that is characterized by hyperproliferation and disrupted differentiation of keratinocytes (Avasatthi *et al.*, 2016; Tripathi *et al.*, 2018). It results in a decrease in apoptotic cell death in keratinocytes and an enhanced resistance of intralesional keratinocytes to apoptosis. Cytokines play an important role in the progression of psoriasis. Major Cytokines include tumor necrosis factor-alpha (TNF-α), Interleukin-23 (IL-23), and IL-17 which aids in the production of other proinflammatory cytokines and psoriasis lesions formation.

The currently available treatments based on the conventional formulation for psoriasis are associated with problems like increased dosing frequency, increased side effects, and decreased safety profile for long term use (Khurana, Arora and Narang, 2018). Among the currently available treatments, none of the psoriasis treatment is found to be safe, effective, and able to completely cure the disease.

To overcome the drawbacks of conventional dosage forms, formulation development is aimed to design drug cargo exhibiting ease of administration, reduced dosing frequency, sustained-release drug, and drug targeting at the required dermal site for improved therapeutic benefit to psoriatic patients (Singh, Kumar and Ahuja, 2005).

Resveratrol (3,5,4′-trihydroxy *trans*-stilbene) is a natural polyphenolic compound, mainly originates from grapes, berries, and red wine. There are several studies on the bioactivities of resveratrol including anti-inflammatory, antioxidant, antimicrobial, and neuroprotective effects.

Due to its inherent antioxidant property, resveratrol has strong chemopreventive as well as antipsoriatic effects (Caddeo *et al.*, 2016). But therapeutic efficacy of resveratrol could not be utilized due to its poor biopharmaceutical properties such as low solubility and poor skin permeation properties (Chedea *et al.*, 2017). Treatment now, thus, aims at higher efficacy through the delivery of drugs in a sustained (temporal) and site-specific (spatial) manner.

The objective of this research was to develop a dermal delivery system of resveratrol for its improved antipsoriatic activity and enhanced dermatological benefits for achieving its enhanced skin deposition profile with limited systemic exposure.

1.1 Skin Drug Delivery

Delivery of drugs to the skin exterior is constantly being explored for skin diseases because it offers a targeted approach (Singh Malik, Mital and Kaur, 2016). Skin delivery is given preference due to various benefits like no systemic toxicity and minimum contact of the drug to the normal tissues (Makhmalzade and Chavoshy, 2018a). Skin, the biggest organ of the human body (1.5 - 2 m^2 surface area), represents the utmost composite barricade between the biological system and the peripheral atmosphere (Waugh and Grant, 2014).

The structure of human skin is a distinctive, well-made membrane and its functions are protecting living organisms from ecological factors and to control water loss from the body. Skin is made up of 3 layers, the epidermis, the dermis, and the hypodermis (subcutaneous tissue) as seen in Figure 1.1 (a).

- The epidermis is also divided into two layers, the stratum corneum, and the viable epidermis.
- The stratum corneum comprising 50 - 150 μm thick consists of corneocytes which are dead, flattened, keratin-rich cells, corneocytes are surrounded by a lipoprotein envelope.
- The corneocytes are embedded in the mixture of intercellular lipids (Abolmaali *et al.*, 2017; Khurana, Arora and Narang, 2018; Nainwal *et al.*, 2019).
- The stratum corneum is an efficient barricade to the passage of molecules with size greater than 500Da, while molecules less than 500Da penetrate with a passive route with intermediate lipophilic and hydrophilic property (log 1–3) (Schlupp *et al.*, 2011).
- It behaves as a rate-limiting barrier for diffusion for almost all drugs due to its structure (Gupta, Agrawal and Vyas, 2012).

Skin delivery of drugs for the treatment of skin disorders is the first choice by most of the patients and physicians which offers many advantages (Degim, 2006) as shown in Figure 1.2. Various skin disorders can be categorized as bacterial infection (such as impetigo, cellulitis) fungal infection (such as sporotrichosis, chronomychosis, blastomycosis), viral diseases (such as herpes simplex virus, eczema), and autoimmune diseases (such as scleroderma, psoriasis). (Nickoloff and Nestle, 2004).

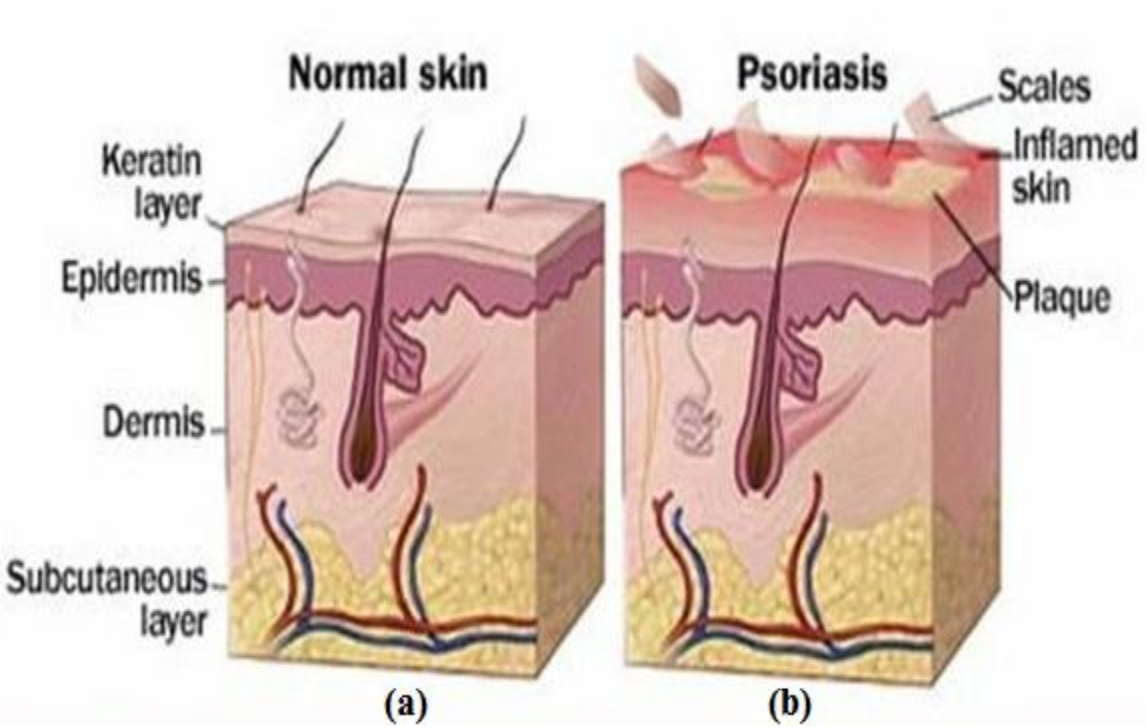


Figure 1.1: Structure of (a) normal skin and (b) psoriatic skin

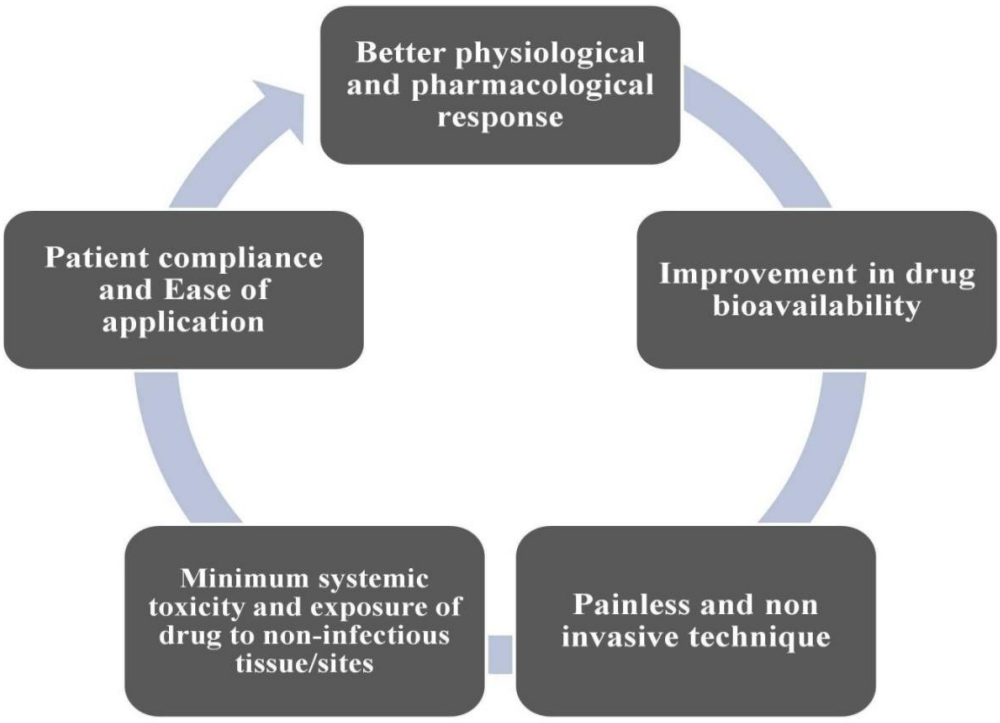


Figure 1.2: Advantages of skin drug delivery

Delivery of drugs to the skin is constantly being explored for several skin diseases because it offers a targeted approach. (Pradhan, Singh and Singh, 2013) However, the barrier in diffusion for most of the drugs represents a major challenge in designing the skin drug delivery system. The various physical and chemical approaches are investigated to overcome the barrier of the stratum corneum. Most of them are associated with disruption of the stratum corneum, (Benson *et al.*, 2019) although few problems associated with conventional skin drug delivery are the high dose of the drug, irritation, and allergic reactions. While, other challenges with conventional skin delivery systems are their stickiness, greasiness, and unappealing texture. Also, changes in skin penetration and its barricade properties during disease skin conditions make it more challenging to construct a competent skin delivery system (Nainwal *et al.*, 2019).

1.2 Psoriasis

Psoriasis is an autoimmune disorder of the skin characterized by relapsing episodes of inflammatory lesions and hyperkeratotic plaques (as shown in Figure 1.1 (b)) with the worldwide occurrence of around 2–5% psoriasis can be classified based on the extent of the inflammatory process, localization of rash, the severity of the patient condition, and other clinical traits into chronic plaque, guttate, pustular, erythroderma, scalp, nail, facial, flexural psoriasis, and psoriatic arthritis as shown in Table 1.1. Amongst these, chronic plaque psoriasis (CPP) represents a major occurrence proportion with the equivalent likelihood in both sexes and early onset before the age of 40 years (Pradhan, Singh and Singh, 2013).

1.2.1 Pathogenesis of psoriasis

Psoriasis is a disease known to be caused by a multitude of both genetic and environmental factors such as trauma, drugs, infection, alcohol, smoking, and stress but its accurate origin is still not known. Unlike normal skin, the pathological progression of psoriasis is supposed to rely on a multitude of coherent events involving the activation of circulating immune cells and their secreted signaling molecules like cytokines, chemokines, and growth factors. These all events, further, progress to mark

hyperkeratosis, congealing of the epidermis, and neovascularization of circulating immune cells and their secreted signaling molecules as shown in Figure 1.3.

Cytokines play an important role in the progression of psoriasis. Major cytokines include tumor necrosis factor-alpha (TNF-α), Interleukin-23 (IL-23), and IL-17 which aids in the production of other proinflammatory cytokines and psoriasis lesions formation (Pradhan, Singh and Singh, 2013).

Several of these pro-inflammatory cytokines e.g. TNF-α, IL-17, and IL-23 rely on nuclear factor kappa B (NF-κB) as a downstream mediator of their effects on a transcriptional level. Accordingly, increased levels of activated NF-κB are found in psoriasis skin compared with healthy skin.

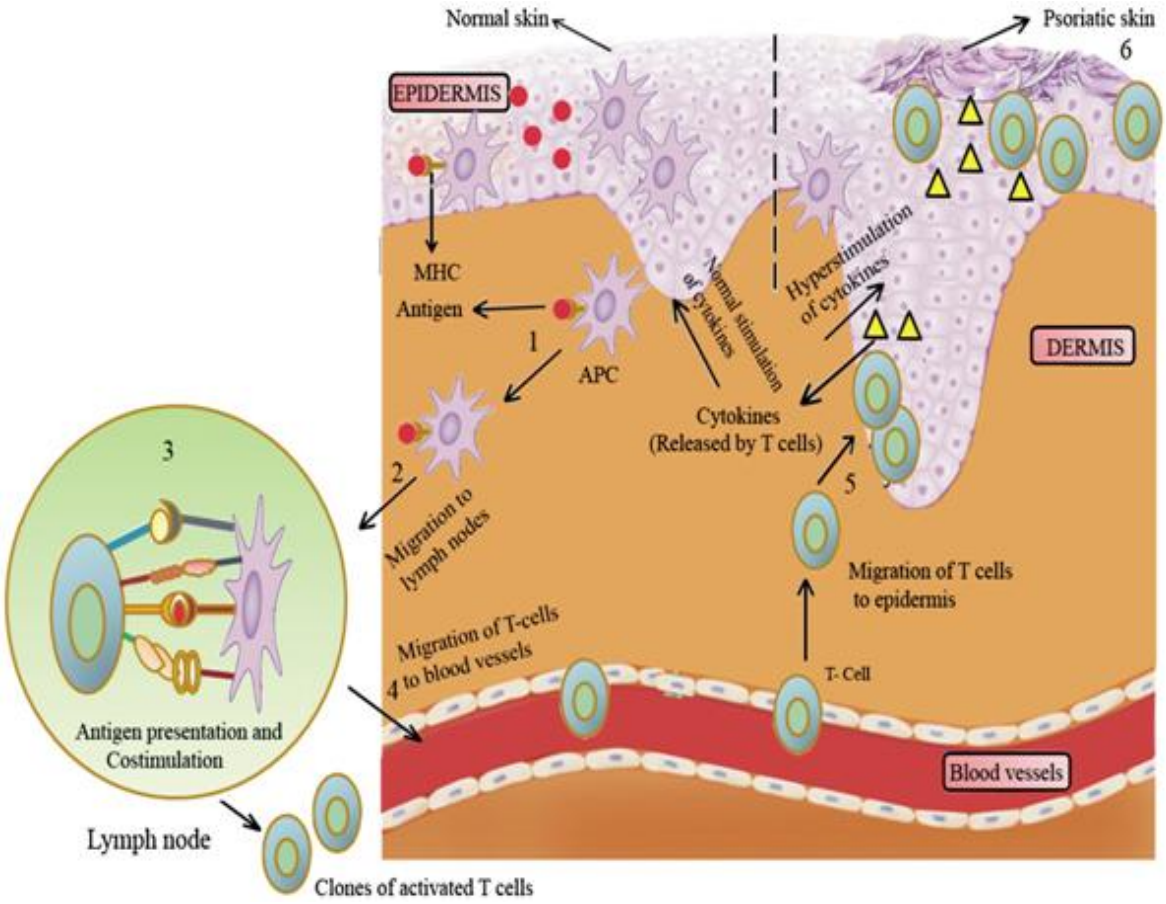
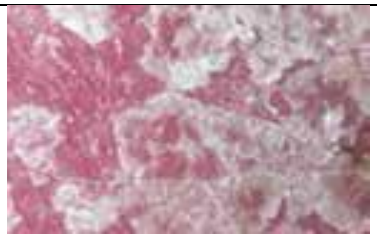


Figure 1.3: Different events in the pathogenesis of psoriasis (Adapted from Pradhan, Singh and Singh, 2016)

Table 1.1: Classification of psoriasis

Plaque psoriasis	Most people have plaque psoriasis. This looks like patches of pink or red skin covered with silvery-white scales (sometimes called plaques). The silvery-white scales are dead skin cells. The patches are slightly raised from the surface of the skin.	
Guttate psoriasis	This looks like lots of small red scaly patches dotted across your skin. These patches can cover quite a large area of your skin.	
Pustular psoriasis	This can be a severe type of psoriasis where lots of small blisters appear on your skin. It needs emergency medical attention.	
Erythrodermic psoriasis	This is a rare and severe type of psoriasis. Most of all of the skin on your body become red and inflamed. It needs emergency medical attention.	
Scalp, nail, facial and flexural psoriasis	Psoriasis can be more difficult to treat in some parts of the body. Flexural psoriasis happens in skin folds, armpits, under the breast, between buttocks, and in the groin area where it can affect the genitals.	 

1.3 Available Treatment Strategies for Psoriasis

Generally, treatment choices for psoriasis involve three main modes namely topical therapy, phototherapy, and systemic therapy. For psoriasis treatment, topical therapies are considered first. Phototherapy is suggested in case of non-effectiveness of topical therapy or relentlessness of the psoriasis condition, which is followed by systemic medications as shown in Table 1.2 (Ahmad Sharabati *et al.*, 2018).

Table 1.2: Available treatment strategies for psoriasis

Type of Therapy	**Class**	**Drugs in Class**
Topical	Tars	Tar
	Anthracene	Dithranol
	Psoralens	Trioxysalen – Methoxsalen
	Others	Fumaric acid – vitamin D (Calcipotriol, Tacalcitol, Calcitriol) – Tazarotene
Phototherapy		Artificial or natural light sources
Systemic	Psoralen	Methoxsalen – Bergapten- Trioxysalen
	Retinoids	Etretinate – Acitretin

Among the currently available treatments, none of the psoriasis treatment is found to be safe, effective, and able to completely cure the disease. Further, available treatment options are related to both inappropriate cosmetic appearance and related toxicities leading to poor patient compliance in long-term use. Phototherapy and systemic agents exhibit numerous ill-effects such as hepatotoxicity, renal toxicity, hypertension, hyperlipidemia, and skin cancer. Moreover, the currently available treatments based on the conventional formulation for psoriasis are associated with problems like increased dosing frequency, increased side effects, and decreased safety profile for long term use. Among the currently available treatments, none of the psoriasis treatment is found to be safe, effective, and able to completely cure the disease. Further, available treatment options are associated with both inappropriate cosmetic appearance and related toxicities

leading to poor patient compliance in long-term use. Therefore improvement of a perfect therapy for psoriasis is a great confront. The lack of effective and safe treatment for psoriasis has created a need to develop and implement a novel approach to make the therapy more useful and acceptable (El-darouti and Hay, 2010). To overcome the drawbacks of conventional dosage forms, formulation development is aimed to design drug cargo exhibiting ease of administration, reduced dosing frequency, and sustained release for improved therapeutic benefit to psoriatic patients.

1.4 Resveratrol as a Potent Antioxidant and Anti-Psoriatic Molecule

Resveratrol (3,5,4′-trihydroxy trans-stilbene) is a naturally occurring polyphenolic compound, mainly originates from berries, grapes, and red wine (Arora and Nanda, 2019). It has been reported to show potent antioxidant, anti-inflammatory, anti-proliferative and anti-cancer effects (Kjær *et al.*, 2015) as represented in Figure 1.4.

Resveratrol is a known activator of sirtuin genes especially SIRT 1 present in the skin which leads to modulation in immune cells and decreases proliferation and promotes differentiation (Nichols and Katiyar, 2011; Carlson *et al.*, 2014; Garcia-Peterson *et al.*, 2017). Along with it, it has inherent antioxidant property, thus it possesses strong chemopreventive as well as antipsoriatic effects (Caddeo *et al.*, 2016).

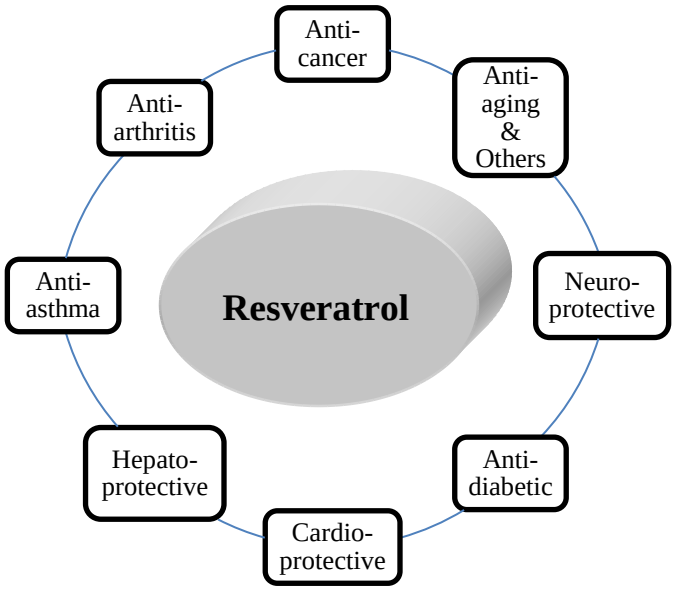


Figure 1.4: Role of Resveratrol in treating several conditions

According to (Lee *et al.*, 2016; Shen and Xu, 2019) "Resveratrol inhibits the protein kinase B pathway by inducing *SIRT1*, leading to cell death, and they indicated resveratrol-mediated activation of *SIRT1* histone deacetylase may be a potential therapeutic target for skin diseases, including psoriasis".

The Resveratrol has a related structure with some endogenous substrates. Thus it can easily interact with enzymes responsible for ROS production (Chedea *et al.*, 2017). Many studies have proved that "Resveratrol reduced the levels of the certain chemokines, downregulated the expression of the proinflammatory cytokines". The anti-oxidative and anti-proliferative mechanism of Resveratrol is well represented in Figure 1.5

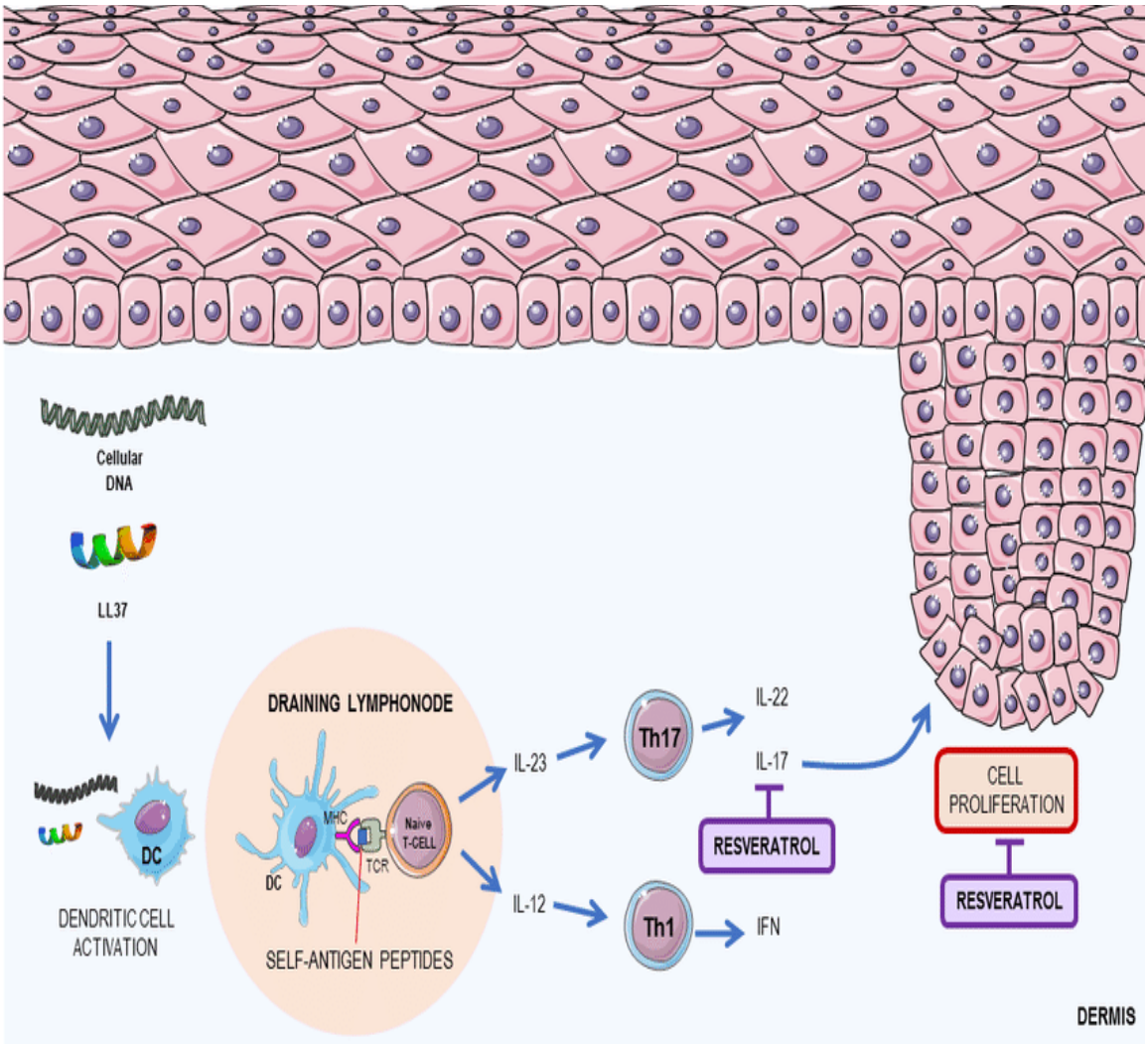


Figure 1.5: Anti-oxidative mechanism of Resveratrol (Adapted from de Brito Oliveira *et al.*, 2017)

1.5 Need for Topical Nanotechnological based Drug Delivery System

Due to the psoriatic skin conditions and allied changes in epidermal anatomy such as hard, inflamed, and thicker keratodermas, and scaly horny barrier, the formulation development for its management is a challenging task. The conventional formulations available in the market are not fulfilling the requirements as they lack the aesthetic and cosmetic properties as well as they show certain toxicity and poor patient compliance in long-term use. Developing novel drug delivery systems with improved dermatological benefits can outweigh the existing challenges. Along with it, they are associated with some other advantages such as ease of administration, reduced dosing frequency, sustained-release drug, and drug targeting at the required dermal site for improved therapeutic benefit to psoriatic patients.

Nanotechnology-based drug delivery system has immense potential to enhance the bioavailability and effectiveness of drugs in their dosage forms, especially lipophilic drugs. For the effective treatment of cutaneous originated disorders such as Psoriasis, the drug should ideally be confined to the surface or within the site of the application without appreciable systemic drug absorption. Thus, the site of application forms the target of topical drug delivery systems. Although topical drug delivery offers certain advantages over systemic delivery for selected drugs and conditions, the resistance against drug transport across the skin barrier remains a major challenge to efficient drug delivery by this route. There is also the potential of skin irritation or contact dermatitis arising from one or more components in the topical formulation. In some cases, poor permeability of some drugs through the skin leads to a poor pharmacological response.

The possibility of allergic reactions and degradation of drugs by the enzymes present in the epidermis may create problems. Rigidization of psoriatic skin has been attributed to a rise in the levels of cholesterol and a fall in the levels of ceramides. Apart from this, normal moisturizing factors (NMFs) like water are almost absent in the psoriatic skin of a patient. As a result of these factors, targeting drug molecules in a vehicle to the psoriatic tissues using the topical route poses a big challenge. A lipid-based carrier system can overcome the lipid imbalance and normal moisturizing factors. The most widely used drug delivery systems include lipid-based nanoparticles i.e. nanoemulsions, solid lipid nanoparticles, lipid nanocapsules, nanosuspension,

liposomes, liquid crystalline nanoparticles, lipid-drug conjugates) or polymer-based nanocarriers (polymeric nanoparticles, polymeric micelles, polymer-drug conjugates) as shown in Table 1.3 (El-darouti and Hay, 2010).

Table 1.3: List of topical novel drug delivery systems for psoriasis

Novel Drug Delivery Systems	AntiPsoriatic Drug	Reference
Liposome-gel	Hydrocortisone	(Kim *et al.*, 1998)
Transfersome in gel	Tacrolimus	(Lei *et al.*, 2013)
Drug β-Cyclodextrine complexes into SLNs	Hydrocortisone	(Cavalli *et al.*, 1999)
Cyclodextrin inclusion complex incorporated in multivesicular liposomes	Fluocinolone acetonide	(Vafaei *et al.*, 2015)
Chitosan coated microemulsion	Methoxsalen	(Behera *et al.*, 2010)
PLGA/PLA nanoparticles (plus zinc)	Betamethasone	(Ishihara *et al.*, 2005)
PLGA (poly(D,L-lactic-co-glycolic acid)) nanoparticles	Betamethasone	(Özcan *et al.*, 2013)
PLGA nanoparticles	Ciclosporine Dexamethasone	(Jain, Mittal and K. Jain, 2011) (Gómez-Gaete *et al.*, 2007)
PLGA microspheres	Clobetasol-17-propionate	(Badıllı, Şen and Tarımcı, 2011)
Poly(ε-caprolactone) (PCL) nanocapsule	Dexamethasone	(Friedrich *et al.*, 2008)
PCL nanoparticles	Hydrocortisone	(Rosado, Silva and Reis, 2013)
Polymeric micelle (PEG-dihexPLA diblock copolymer)	Ciclosporine	(Lapteva, Santer, *et al.*, 2014)
Poly(NIPAM-co-BA) nanogel	Methotrexate	(Singka *et al.*, 2010)
Methoxy-poly(ethylene glycol)-dihexyl substituted polylactide (MPEG- dihexPLA) diblock copolymer micelle	Tacrolimus	(Lapteva, Mondon, *et al.*, 2014)
Niosome	Anthocyanin complex	(Priprem *et al.*, 2015)

	Dithranol Methotrexate	(Agarwal, Katare and Vyas, 2001) (Abdelbary and Aboughaly, 2015)
Lecithin/chitosan nanoparticles	Betamethasone Clobetasol-17-propionate	(Özcan *et al.*, 2013) (Şenyiğit *et al.*, 2010)
Liposome	Tacalcitol Clobetasol-17-propionate Dithranol Tacrolimus Tretinoin Triamcinolone	(Körbel *et al.*, 2001) (Rao and Murthy, 2000) (Abdelbary and Aboughaly, 2015) '(Kumar, Deep and Agarwal, 2015) (Özcan *et al.*, 2013) (Raza, Singh, Singla, *et al.*, 2013) (Yu and Liao, 1996)
Pegylated liposomes	Calcipotriol	(Knudsen *et al.*, 2012)
Transferosome	Dexamethasone Hydrocortisone Triamcinolone	(Cevc and Blume, 2004) (Fesq *et al.*, 2003)
Flexible vesicles and conventional vesicles	Cyclosporine	(Guo *et al.*, 2000)
Deformable liposomes	Methotrexate	(Srisuk *et al.*, 2012) (Trotta *et al.*, 2004)
Ethosome	Ciclosporine Methotrexate Psoralen Tacrolimus Tretinoin Tretinoin	(Verma and Fahr, 2004) (Dubey *et al.*, 2007) (Zhang *et al.*, 2014) (Li *et al.*, 2012) (Raza, Singh, Lohan, *et al.*, 2013)
SLNs (Solid lipid nanoparticles)	Betamethasone Ciclosporine Clobetasol-17-propionate Dithranol Methoxsalen. Mometasone furoate Prednicarbate Psoralen Tretinoin Triamcinolone	(Zhang and Smith, 2011) (Kim *et al.*, 2009) (Kalariya *et al.*, 2005) (Gambhire, Bhalekar and Gambhire, 2011) (Battaglia *et al.*, 2012) (Madan, Dua and Khude, 2014) (Raza, Singh, Lohan, *et al.*, 2013) (Schlupp *et al.*, 2011) (Fang *et al.*, 2008) (Pradhan, Singh and

		Singh, 2016) (Sonawane *et al.*, 2014)
SLN-hydrogel	Betamethasone dipropionate and calcipotriol Halobetasol Tacrolimus	(Bikkad *et al.*, 2014) (Ruihua Wang, 2012) (Lin *et al.*, 2010)
Nanostructured lipid carriers (NLCs)-hydrogel	Acitretin	(Antônio Dantas Silva *et al.*, 2012)
NLCs	Calcipotriol and methotrexate Clobetasol-17-propionate Fluocinolone acetonide Methotrexate Methoxsalen Psoralen Tacrolimus Tretinoin	(Pradhan, Singh and Singh, 2015) (Pinto *et al.*, 2014) (Nam, Ji and Park, 2011) (Baboota *et al.*, 2011) (Marepally *et al.*, 2014) (Sonawane *et al.*, 2014) (Raza *et al.*, 2011) (Raza, Singh, Singla, *et al.*, 2013)
Microemulsions in hydrogel	Betamethasone dipropionate and salicylic acid Methoxsalen	(Baroli *et al.*, 2000) (Lei *et al.*, 2013)
Microemulsion	Dithranol Methoxsalen Tacrolimus	(Sah, Jain and Pandey, 2011) (Shinde *et al.*, 2013) (Wohlrab *et al.*, 2012)
Nanoemulsion	Methoxsalen	(Vicentini *et al.*, 2013)
Liquid crystalline nanoparticles	Cyclosporine A Tacrolimus	(Vicentini *et al.*, 2013) (Thapa and Yoo, 2014) (Petrilli *et al.*, 2016)

The categorical representation of different kinds of nanocarriers for topical skin delivery is depicted in Figure 1.6.

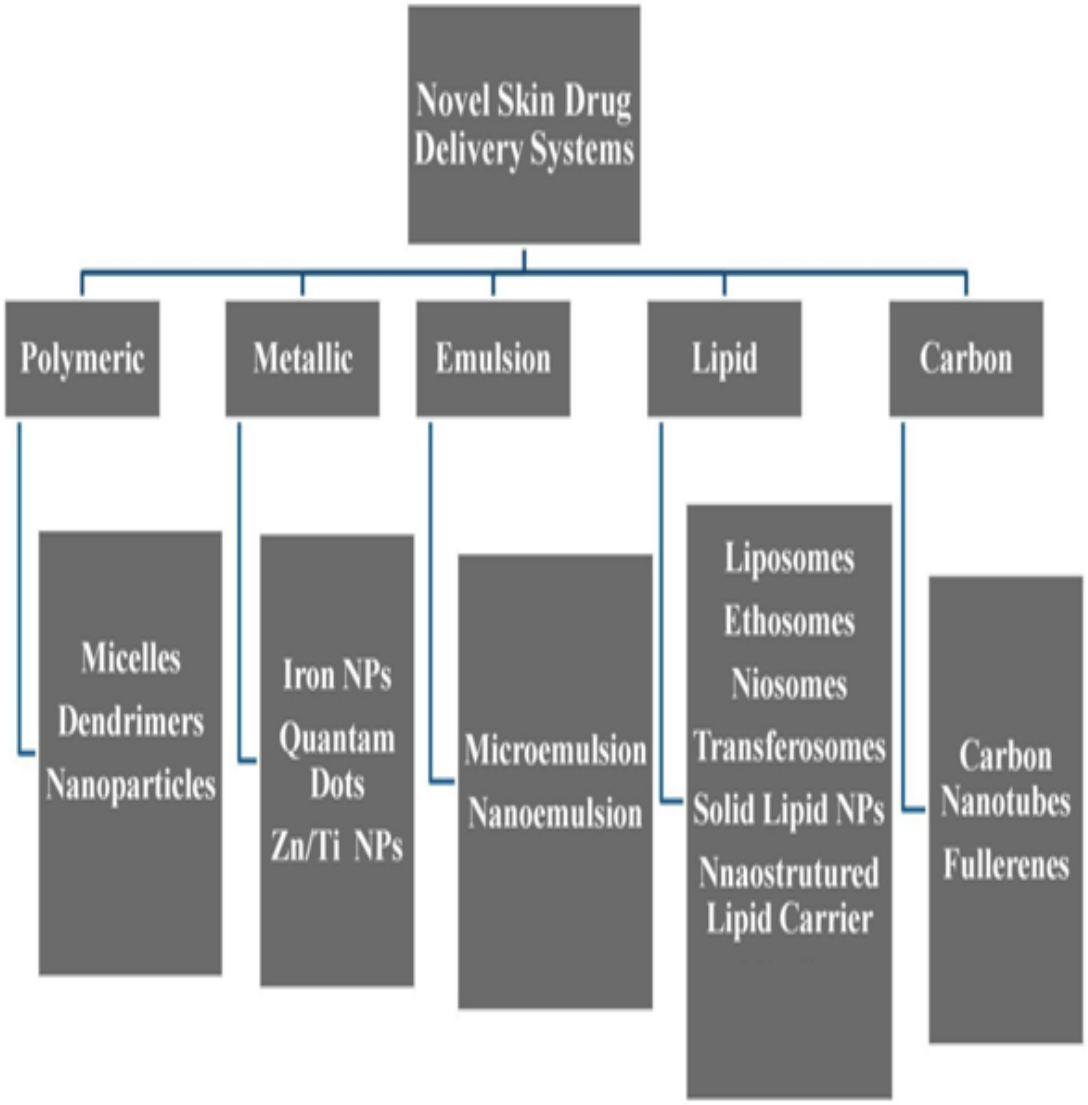


Figure 1.6: Categorical representation of different kinds of nanocarriers for topical skin delivery

Among many nanosystems attempted for dermatological benefits, micellar drug delivery systems and nanoemulsion are a few of the successful approaches having better skin permeation properties, skin compatibility, and high stability.

1.6 Polymeric Micelles

Polymeric micelles are colloidal carriers which are nano-sized assemblies and are composed of amphiphilic block polymers and characterized by core-shell morphology

formed through self-association of hydrophilic and hydrophobic block copolymers in aqueous media as shown in Figure 1.7 (Makhmalzade and Chavoshy, 2018b).

Polymeric micelles that consist of amphiphilic copolymers in the form of core/shell nanostructures could be exploited for the delivery of both hydrophilic and hydrophobic drugs. These comprise an inner core and an outer shell, where the drug can be incorporated according to their nature. The ability of the micelle to solubilize lipophilic compounds directly depends on the nature and the size of the hydrophobic moiety of the copolymer as depicted by Figure 1.8.

The nature of polymer can influence the intermolecular interactions between the drug and the polymer and the size will affect the amount of drug that can be incorporated. Micellar preparation methods and process-specific parameters (such as the structure of the organic solvent, solvent ratio) affect the drug loading capacity. The unique feature that has made polymeric micelles superior to other colloidal delivery systems is the versatility of the core/shell structure and is considered as a promising drug carrier for the effective treatment of various skin diseases (Lee, Shenoy and Sheel, 2010).

Micelles based on the single polymer are known to face several drawbacks such as large particle size, less stable, low drug-carrying capacity, high CMC, etc, (Ebrahim Attia *et al.*, 2011) while mixed micelles show synergistic properties. Widely used amphiphilic triblock copolymers are Pluronics which are made of hydrophilic polyethylene oxide (PEO) and hydrophobic polypropylene oxide (PPO) blocks (PEO–PPO–PEO).

Pluronic F127 (PEO_{100}–PPO_{69}–PEO_{100}) is known to have a high HLB value (approximate 22) and a monomer unit ratio of 100/69/100. Owing to the higher proportionate of PEO and long hydrophilic chains, it is quite more hydrophilic with a higher CMC value and yields kinetically stable micelles produced by steric hindrance. While Pluronic P123 (PEO_{20}–PPO_{65}–PEO_{20}) have less HLB value (approximate 7-9) yield more thermodynamically stable micelles because of more tight hydrophobic interactions of PPO chains. Thus, mixed pluronic micelles were developed (Abd-Elsalam, El-Zahaby and Al-Mahallawi, 2018).

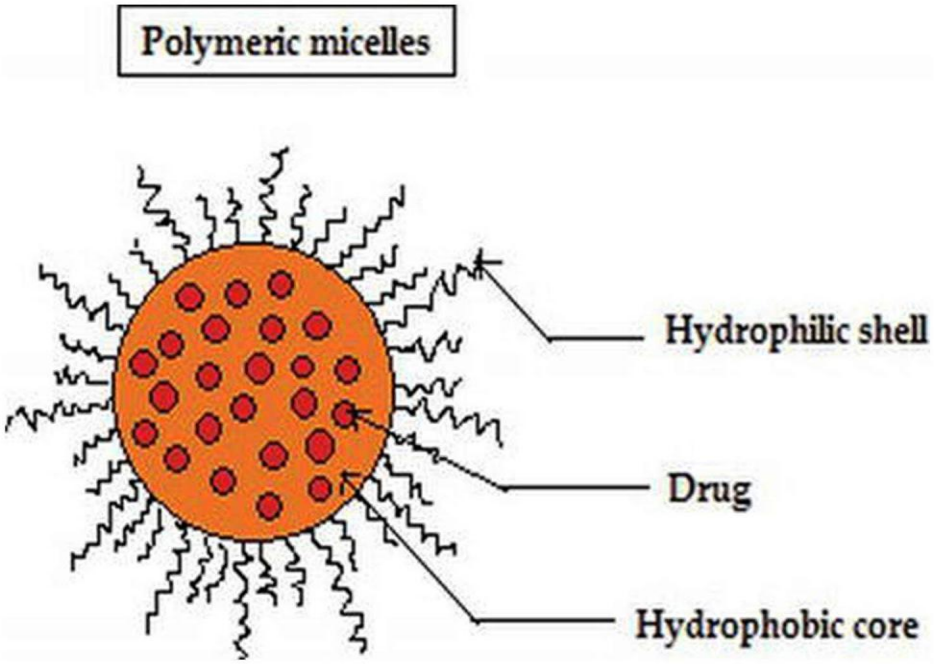


Figure 1.7: Core-shell morphology of polymeric micelles

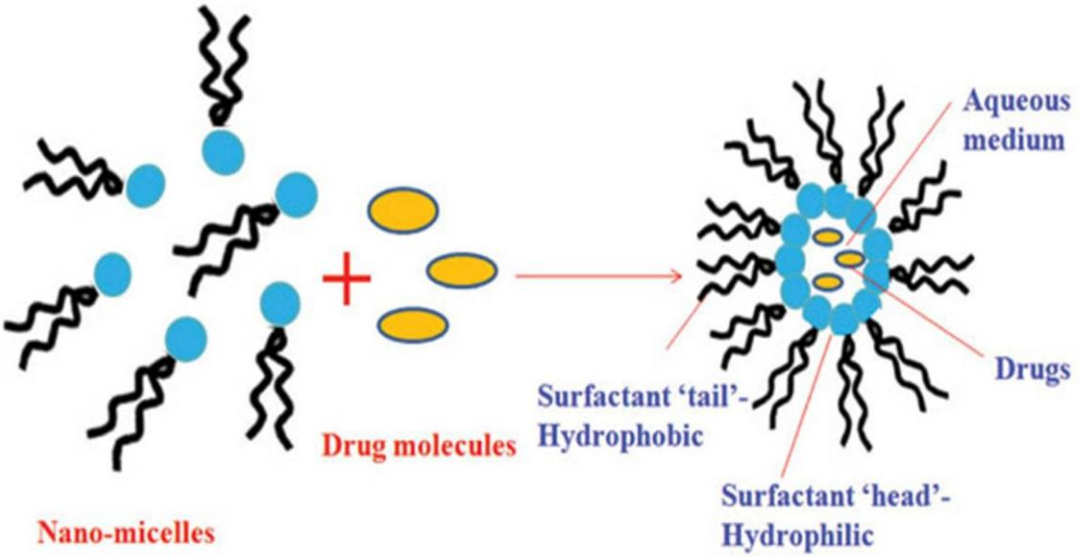


Figure 1.8: Formation of polymeric micelles

1.7 Nanoemulsion

Nanoemulsions are emulsions with droplet size on the order of 100 nm. A typical nanoemulsion contains oil, water, and an emulsifier. Nanoemulsions can be rendered into several dosage forms, like liquids, creams, sprays, gels, aerosol, and foams; and can be administered by equally varying routes like topical, oral, intravenous, intranasal, pulmonary, and ocular. They possess higher solubilization capacity than simple micellar dispersions, greater kinetic stability than coarse emulsions, and have found use in the cosmetic industry (Nickoloff and Nestle, 2004). Their long-term physical stability is a direct consequence of small droplet size, which impairs conventional destabilization phenomena like creaming, sedimentation, and coalescence. When administered topically, mini scale size of droplets in nanoemulsion and their capability to solubilize very hydrophobic drugs provides a pathway to drastically increase the rate of drug dissolution and subsequently expected systemic bioavailability through the transcellular route.

Drug release from nanoemulsion involves the partitioning of it from oil into the surfactant layer and then into the aqueous phase, thus avoids occlusive effects (Jaiswal, Dudhe and Sharma, 2015). Various methods to develop nanoemulsion are depicted in Figure 1.9.

1.7.1 Components in the topical nanoemulsion formulations for antipsoriatic drugs

1.7.1.1 Oil phase

Selection of oil phase such as saturated and unsaturated fatty acids/fatty acid esters like castor oil, coconut oil, corn oil, cottonseed oil, evening primrose oil, fish oil, jojoba oil, lard oil, linseed oil, mineral oil, olive oil, peanut oil, PEG-vegetable oil, perfluorochemicals, pine nut oil, safflower oil, sesame oil, soybean oil, squalene, sunflower oil, wheat germ oil can be done.

The oil phase helps in the penetration of drugs. Mostly antipsoriatic drugs are lipophilic and have a log P value of 3, which makes it suitable for being encapsulated in the emulsion. Sometimes a combination of oils is used to encapsulate antipsoriatic drugs (Somagoni *et al.*, 2014).

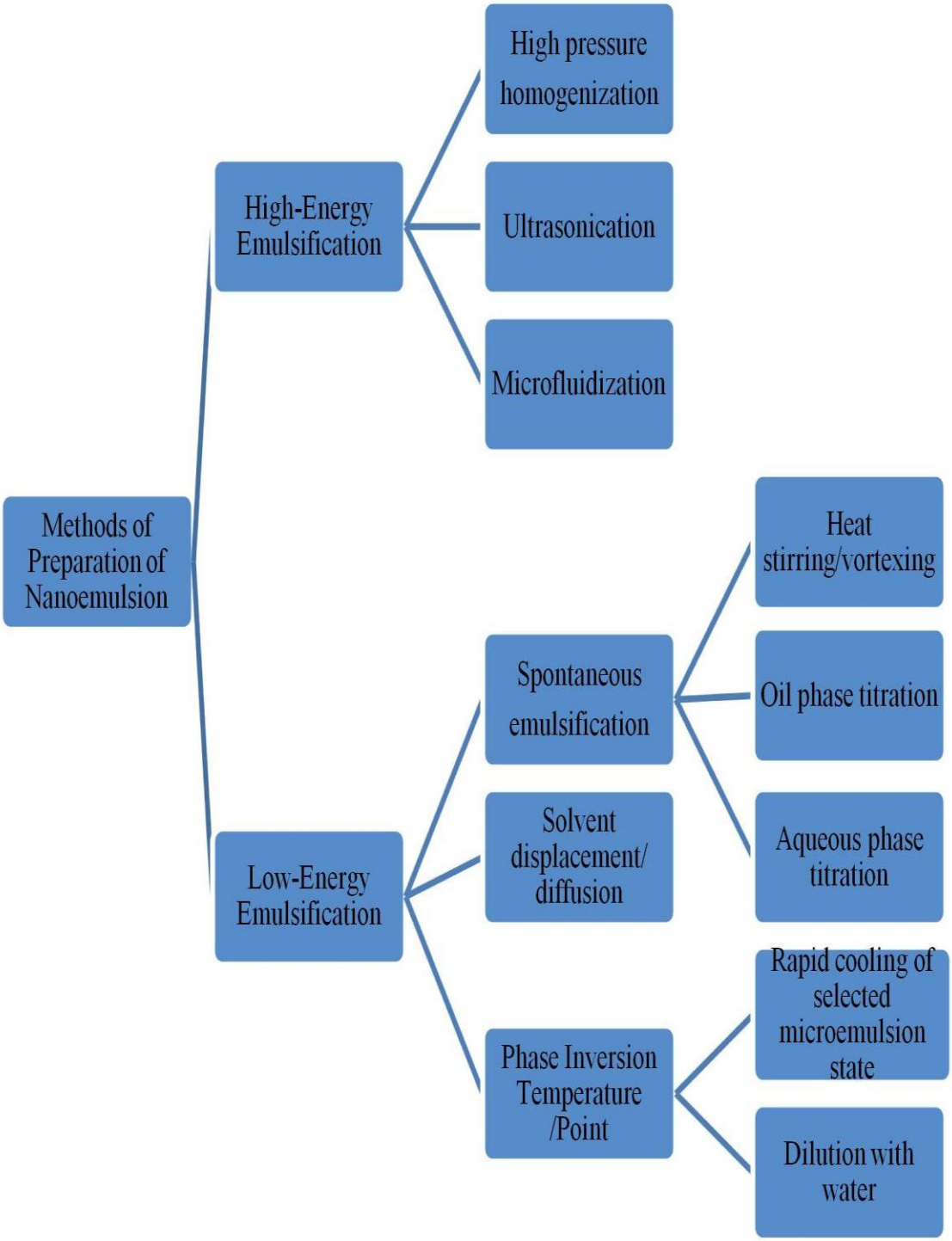


Figure 1.9: Various methods for the preparation of nanoemulsion

1.7.1.2 Surfactant

Selection of surfactant is used to decrease the interfacial tension and makes a stable emulsion having requisite particle size, but which also ensures minimal skin irritancy there are four types of surfactants i.e. nonionic, zwitterionic cationic, anionic.

Commonly used surfactants include Tween®, Cremophor®, Transcutol® P, Plurol Oleique®, Plurol Isostearique®, and Labrasol®, Lecithin, Organogels (Kaur *et al.*, 2017).

1.7.1.3 Co-surfactant

Cosurfactants are generally used to modify the curvature and fluidity of the interfacial film, leading to a decrease of interfacial tension. Cosurfactants are short and medium-chain alcohols and polyglyceryl derivatives, including ethanol, isopropanol, isopropyl myristate, and propylene glycol (PG) (Somagoni *et al.*, 2014).

1.7.1.4 Other Excipients

Antioxidants (a-tocopherol, ascorbic acid) Tonicity modifiers (glycerol, sorbitol, xylitol) pH adjustment agents (NaOH or HCl) Preservatives, aqueous phase (sodium chloride and buffer salts) and penetration enhancers, Viscosity enhancing agents (e.g., Carbopol®, Aerosil®, gelatin) are incorporated to reduce the fluidity and generate the desired final consistency of the product (Salim *et al.*, 2016).

1.7.2 Stability of nanoemulsion

Emulsion stability is dependent on the role of surfactants, its composition, and the drop size distribution. Major instability is due to coalescence in which there is a fusion of droplets by breaking the film between the two globules which in turn increases the size of nanoemulsion.

It can be prevented by adding a sufficient amount of surfactant while preparing nanoemulsions. Another one is Ostwald ripening in which emulsions get destabilized with time due to molecular diffusion and change in droplet size of nanoemulsion. There is mass transfer occurs from the dispersed phase to the continuous phase (Sonal Setya, Sushama Talegaonkar, 2014).

1.8 Quality by Design (QbD) and Design of Experiments (DoE) based Optimization and Development of Nanocarriers Systems

The development of such complex nanocarriers involves the combination of several diverse material and process variables. To achieve the desired product profile of target formulation and to achieve the prerequisite quality characteristics of the developed drug product, the formulation scientist needs to optimize all the associated variables systematically as suggested by ICH Q8 (R2) guideline for product development. Traditional one variable at a time (OVAT) approach of optimizing drug delivery does not solve the purpose completely, while a more systematic multivariate response surface methodology is preferred nowadays as per regulatory requirements also.

Thus, the Quality by Design (QbD) approach has a pivotal role in formulation optimization and development which states that it is "a systematic approach of development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management" (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, 2009; Saydam and Takka, 2018).

Initially, quality by test (QbT) was the primary approach to assure the quality of drug products which was based on methods without a clear understanding of the processes. A schematic difference between QbT and QbD is depicted in Figure 1.10.

QbD driven Design of Experiment (DoE) endows with a holistic perception of products and processes to relent the best solution. "The conventional system of optimizing the drug delivery system involves studying the power of one variable at a time (OVAT) while keeping others as constant". However by applying this conventional method, the explanation of a definite challenging objective can be accomplished partially, but the accomplishment of the best possible formula, concentration/ amount of constitutive ingredients, or process is never guaranteed. "Establishment of "cause-and-effect" relationships using OVAT is not possible and it becomes ineffective when all variables are changed simultaneously" (Kharia and Singhai, 2013). Thus more systematic optimization approaches are need of the hour to avoid such inconsistencies and to produce competent formulation under a strictly regulated environment.

The basic elements and tools of QbD approach such as identifying Quality Target Product Profile (QTPP), Critical Quality Attributes (CQAs), Critical Material Attributes (CMAs), and Critical Process Parameters (CPPs) can be employed along with the application of DoE to establish the relationship between CQAs and CMAs.

Several tools of common risk analysis and management are employed such as Fishbone diagrams and failure mode and effect analysis (FMEA) application. Design of experiments and multivariate statistical data analysis are indispensable fundamentals of QbD, accepted by recent International Conference of Harmonization Q8 guideline.

The Design of Experiment is one of the most important tools of optimization. Response surface methodology is utilized for systematic optimization by employing several experimental designs such as Factorial Design, Central Composite Design, Box-Behnken Design, etc.

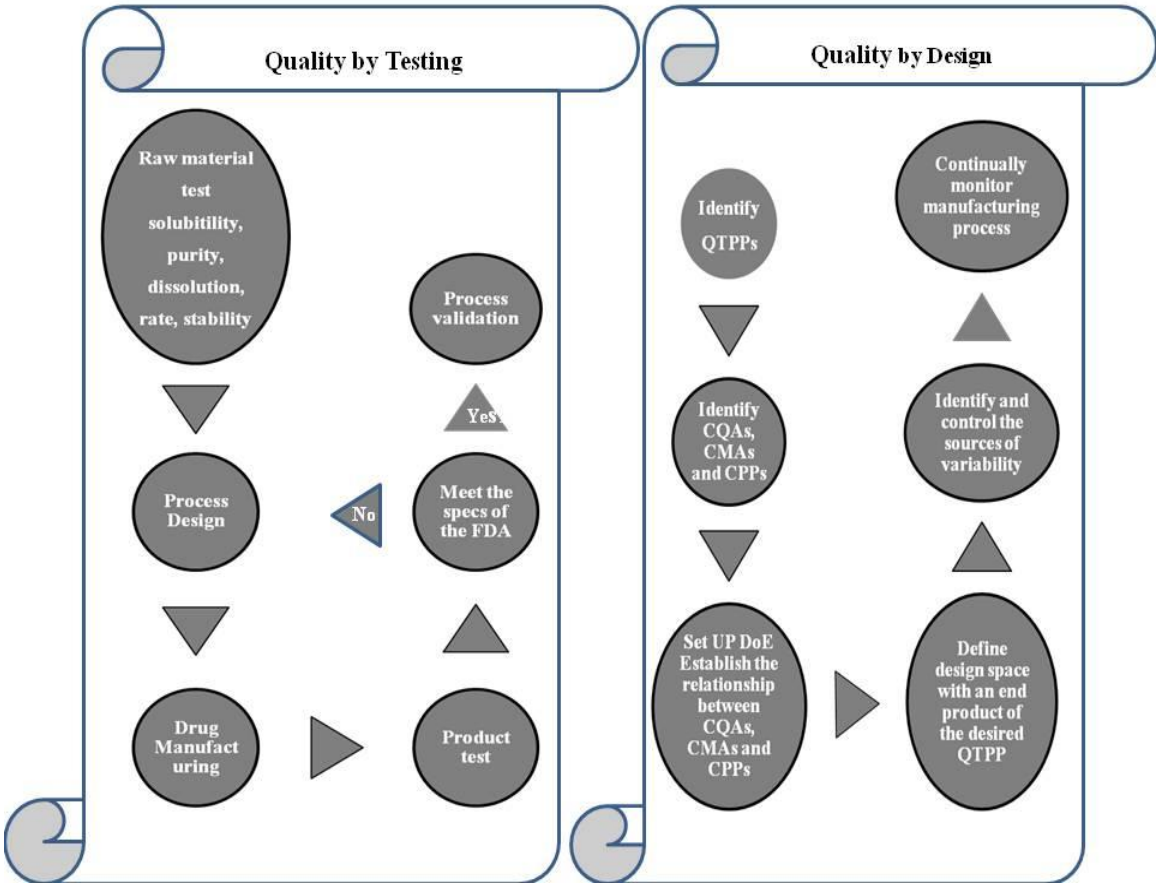


Figure 1.10: Difference between QbT and QbD

1.9 Research Envisaged

Psoriasis is a chronic inflammatory disorder that is particularly characterized by abnormal proliferation and disrupted differentiation of keratinocytes along with reverse occurrence of inflammatory lesions and neovascularization. Being an autoimmune disease, it is manifested by immunological and biochemical alteration and thus activates immune cells. Thus, it is associated with increased levels of pro-inflammatory markers, chemokines, growth factors, and cytokines such as TNF-α, IL-17, and IL-23. Cytokines play an essential role in the pathophysiology of psoriatic like a skin condition. Among its various types "Plaque psoriasis" is the most prevalent type affecting approximately 3% of the total population as it is associated with red or pink patches on the surface of skin covered by whitish or silver scales (known as plaque).

Though it does not affect the overall health of the diseased person nor has any risk of mortality, however, it produces pessimistic effects on quality of life as it is associated with social dishonor, shame, state of grief, and physical disability which results in physical and psychosomatic shock on patients' health. The etiology of psoriasis is unknown and majorly linked with autoimmunity and genetic reasons.

The treatment options include topical therapy, phototherapy, and systemic therapy. For severe cases, systemic therapy along with topical therapy is recommended. Systemic therapy includes corticosteroids, immunosuppressant drugs such as cyclosporine or retinoids. The use of these drugs systemically or topically results in much severe toxicity like renal and hepatic toxicity, etc as well as patient noncompliance. Among all the available treatments, none approach can cure completely, safely, and effectively.

Resveratrol (3,5,4′-trihydroxy trans-stilbene) is a naturally occurring polyphenolic compound, mainly originates from berries, grapes, and red wine. It has been reported to show potent antioxidant, anti-inflammatory, anti-proliferative and anti-cancer effects.

Resveratrol is a known activator of sirtuin genes especially *SIRT1* present in the skin which leads to modulation in immune cells and decreases proliferation and promotes differentiation. Along with it, it has inherent antioxidant property, thus it possesses strong chemopreventive as well as antipsoriatic effects. However, Resveratrol is a natural

originated molecule but is therapeutic efficacy could not be utilized due to its poor biopharmaceutical properties such as low solubility and poor systemic bioavailability.

Treatment now, thus, aims at higher efficacy through the delivery of drugs in a sustained (temporal) and site-specific (spatial) manner. Topical skin delivery is the most ideal and route of choice for several dermatological disorders including psoriasis. But due to the psoriatic skin conditions and allied changes in epidermal anatomy such as hard, inflamed, and thicker keratodermas, and scaly horny barrier, the formulation development for its management is a challenging task. The conventional formulations available in the market are not fulfilling the requirements as they lack the aesthetic and cosmetic properties as well as they show certain toxicity and poor patient compliance in long-term use. The stratum corneum acts as the main barrier and challenge to be overcome for the permeation of the bioactive molecule. Overcoming this horny barrier by enhancing the permeation and at the same time, retaining the maximum amount of drug in deeper layers, and thus, avoiding systemic absorption to enhance the dermatological benefits via topical route is the need of the hour.

To overcome the drawbacks of conventional dosage forms, formulation development is aimed to design drug product exhibiting ease of administration, higher penetration, reduced dosing frequency, and sustained release for improved therapeutic benefit to psoriatic patients.

Developing novel drug delivery systems with improved dermatological benefits can outweigh the existing challenges. Along with it, they are associated with some other advantages such as ease of administration, reduced dosing frequency, sustained-release drug, and drug targeting at the required dermal site for improved therapeutic benefit to psoriatic patients.

In the past few decades, several lipids and polymeric novel topical drug delivery systems have been exploited to overcome the barriers of topical skin delivery of drugs and bioactive

Among many nanosystems attempted for dermatological benefits, micellar drug delivery systems and nanoemulsion are a few of the successful approaches having better skin permeation properties, skin compatibility, and high stability.

The present work was focused on the formulation and evaluation of Resveratrol loaded polymeric micelles (PM) and lipidic based nanoemulsion (NE) delivery systems and their corresponding hydrogels (PMG and NEG). The delivery systems were formulated using QbD and systematic optimization (DoE) based approach.

The proposed systems are novel approaches for formulating Resveratrol containing delivery system for the treatment of Plaque Psoriasis. The developed system was evaluated for *in vitro* skin permeation abilities as well as *in vivo* antipsoriatic efficacy. It is envisaged that these novel delivery systems may follow maximum pathways of skin absorption and hence, may provide a better therapeutic effect as compared to the conventional delivery system as depicted in Figure 1.11.

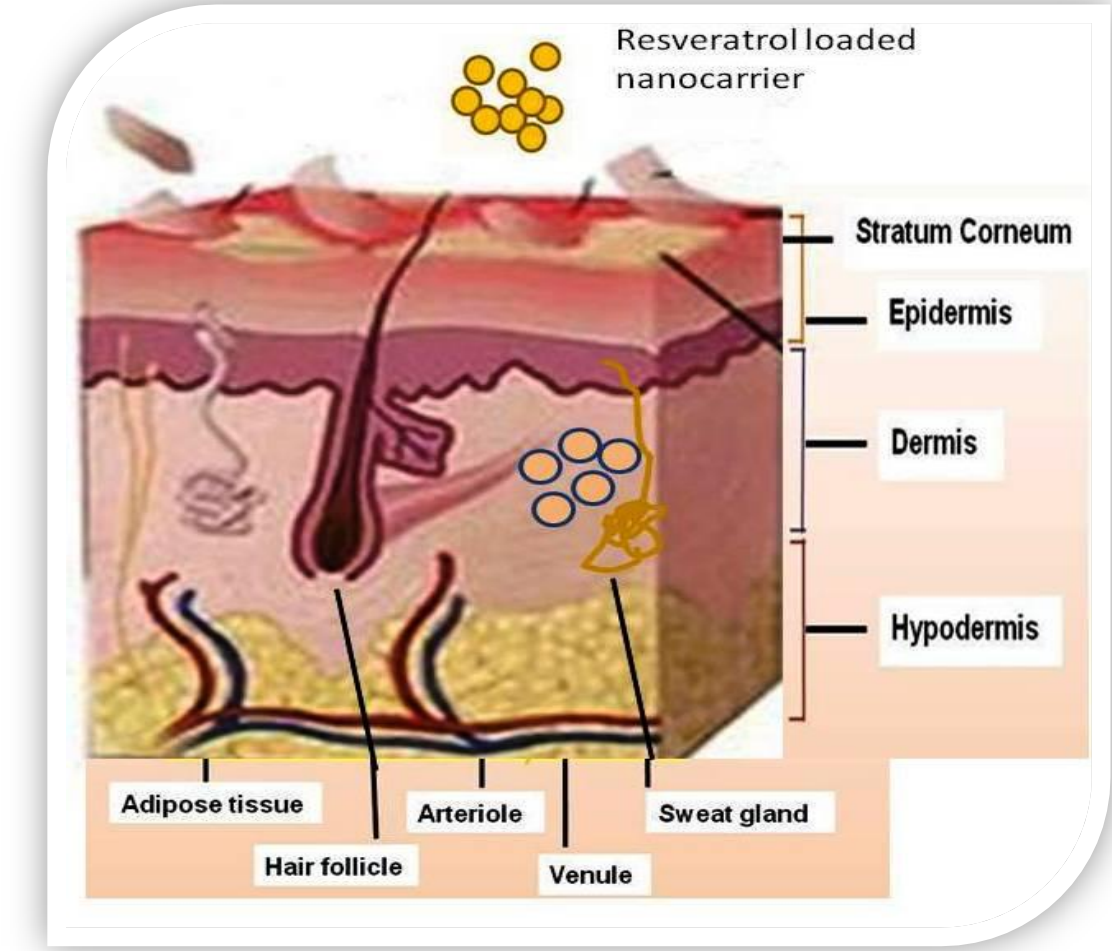


Figure 1.11: Diagrammatic representation showing research envisaged

1.10 Research Aim and Objectives

In the present research, encapsulation of potent antipsoriatic agents i.e. Resveratrol into nanocarriers with increased skin penetration ability was envisaged. Colloidal drug carriers i.e. polymeric micelles and nanoemulsion are the best-suited nano delivery systems to encapsulate and deliver Resveratrol successfully due to their inherited properties for the safe and effective delivery of the antipsoriatic agent.

The main aim of the current study is to design, develop, and evaluate these delivery systems containing Resveratrol for its better antipsoriatic activity.

Objectives:

- To develop nanoemulsion and polymeric micelles based topical formulation.
- *In vitro* characterization and optimization of with respect to different process and formulation variables.
- *In vitro* skin permeation and deposition studies in comparison to conventional formulation(s).
- To evaluate antipsoriatic activity of the developed formulation(s).
- To perform accelerated stability studies of the developed formulation as per ICH guidelines.

1.11 Plan of Work

1. **Extensive Literature Survey**

2. **Preformulation studies of bioactive (Resveratrol)**
   - Identification studies using UV Spectroscopy and HPLC studies
   - Melting point analysis and Purity studies using FTIR Spectrum and DSC
   - Partition Coefficient analysis
   - Solubility studies

3. **Development of the analytical method for the determination of Resveratrol using UV spectroscopy and HPLC**

4. **Optimization and formulation of polymer-based delivery system i.e. polymeric micelles (PM) and its corresponding hydrogel (PMG)**
   - Development and optimization of polymeric micelles using Quality by Design approach employing Central Composite Design
   - Characterization of developed colloidal micellar formulation
     - *Size, polydispersity index, and zeta potential*
     - *Morphological evaluation (TEM)*
     - *Micellar incorporation efficiency*
   - Formulation of carbomer based hydrogel embedded with colloidal micelles (PMG)

5. **Optimization and formulation of lipid-based delivery system i.e. Resveratrol loaded Vitamin E based nanoemulsion (NE) and its corresponding hydrogel (NEG)**
   - Development and optimization of Resveratrol loaded vitamin E based nanoemulsion using full factorial design
   - Formulation of carbomer based nanoemulsion hydrogel (Nanoemulgel)
   - Characterization of developed Nanoemulsion and nanoemulgel

- *Globule size, zeta potential, and PDI*
- *Surface morphology via Transmission electron microscopy*
- *Resveratrol content in nanoemulgel*

6. **Some other important evaluations of formulated Micellar hydrogel and Nanoemulgel**
   - Determination of pH
   - Spreadability studies
   - Rheological behavior and viscosity
   - *In vitro* Resveratrol release studies
   - Skin permeation and deposition profile (*ex vivo*)
   - Skin targeting studies: Histopathology by CLSM
   - *In vitro* antioxidant activity using DPPH Assay

7. ***In vivo* efficacy studies (antipsoriatic activity) using IMQ-induced psoriatic-like plaque model in Swiss Albino Mice**
   - Psoriasis Area and Severity Index (PASI) score
   - Cytokines level in serum and spleen dimension & weight
   - Histology of skin

8. **Stability Studies of optimized formulations**
   - Evaluation of the effect of different storage conditions
     - *Refrigerated temperature (5 ± 3 ºC)*
     - *Room temperature (25 ± 2 ºC/ 60 ± 5 % RH)*

9. **Statistical Analysis**

10. **Thesis Compilation, the publication of research outcomes, and thesis submission**